II. REMARKS

Upon entry of the present amendment, claims 1 to 3, 5, 13, 14, 20, and 40 to 45 will be pending.

A. Regarding the Amendments

The specification has been amended to substitute the embedded hyperlinks with language referring to the previously recited links. As such, the amendments merely address a formality, and do not add new matter.

Claim 4 and, pursuant to the restriction requirement, claims 6 to 12, 15 to 19 and 21 to 39 are cancelled herein without disclaimer, and without prejudice to Applicants' pursuing prosecution of subject matter encompassed within one or more of the claims in an application claiming the benefit of priority of the subject application.

Also pursuant to the restriction requirement, claims 1 to 5, 20, and 40 to 42 have been amended generally to refer to a transgenic or chimeric non-human "mammal", which contains a transgene comprising "a truncated Activin Type II receptor". As such, the amendments merely address a formality, and do not add new matter.

Claims 1 and 40 to 42 also have been amended to more clearly indicate that a truncated Activin Type II receptor "lacks kinase activity". The amendment is supported, for example, by original claim 4, which is cancelled herein, and at paragraph 115 (page 43), paragraph 164 (page 63), and paragraph 304 (pages 109-110). As such, the amendment does not add new matter.

Claim 1 also has been amended to delete a duplicate occurrence of the term "corresponding". Claim 3 has been amended to more fully recite the terms "Activin RIIA" and "Activin RIIB" in order to clarify the claimed subject matter and to provide the requisite antecedent basis for claim 4, which depends from claim 3. Claim 5 has been amended to delete the term "receptor", which is redundant with the abbreviation "R" in the term "Activin RIIB". As

such, the amendments merely correct a typographical error or address formalities, and do not add new matter.

Claim 42 has been amended to include "ovine" transgenic mammals, and to clarify that the embryos are implanted into a pseudopregnant female of the same species. The amendments are supported, for example, at paragraph 142 (pages 54-55) and paragraph 204 (page 77) and, therefore, do not add new matter.

New claims 43 to 45 have been added. The new claims are supported, for example, at paragraph 142 (pages 54-55) and, therefore, do not add new matter.

B. Regarding the Restriction Requirement

Pursuant to the Restriction Requirement, the non-elected claims have been cancelled without prejudice.

C. Regarding the Priority Claim

It is stated in the Office Action that the priority applications fail to provide literal or figurative support to the use of activin receptors in the construction of non human transgenic animals. Applicants point out, however, that U.S. Serial No. 09/626,896, filed July 27, 2000, clearly discloses transgenic animals comprising a dominant negative Act RIIB receptor (page 82, line 30, to page 83, line 12) and provides methods of making such a transgenic animal (see Example 11, pages 111-118, particularly page 115, line 22, to page 116, line 22). As such, the subject application clearly is entitled to the July 27, 2000, filing date of U.S. Serial No. 09/626,896.

Further, U.S. Serial No. 09/485,046, filed January 31, 2000, which is a National Stage application of PCT/US98/15598, filed July 28, 1998, discloses a similar phenotype between activin receptors, specifically Act RIIB, knock out mice and the GDF-11 knock out mice, indicating that Act RIIB can act as a GDF-11 receptor (page 37, lines 2-4; page 43, lines 6-14).

Further, these priority applications disclose that peptide fragments of a GDF receptor can be used to inhibit binding of GDF-8 or GDF-11 to its receptor (page 3, lines 1-4; see, also, page 25, lines 5-15, and page 26, lines 1-7). As such, it is submitted that the claimed invention is entitled to the July 28, 1998, filing date of the PCT application.

D. Regarding the Specification

The specification is objected to as containing embedded hyperlinks and/or other forms of browser-executable code. The specification has been amended to address this informality. As such, it is respectfully requested that this objection to the specification be withdrawn.

E. Claim Objections

Claim 1 to 5, 13, 14, 20 and 40 to 42 are objected to as being broader than the elected invention, and claim 1 is objected to as reciting the term "corresponding" twice. The claims have been amended to delete reference to the non-elected subject matter, and to delete the second occurrence of the term "corresponding", respectively. As such, it is respectfully requested that the objections to the claims be withdrawn.

F. Rejections under 35 U.S.C. § 112

The objection to the specification and corresponding rejection of claims 1 to 5, 13, 14, 20 and 40 to 42 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement is respectfully traversed.

It is acknowledged in the Office Action that the specification is enabling for a transgenic mouse whose genome contains a nucleic acid sequence encoding a truncated Activin IIB receptor ("ActRIIB"), specifically a truncated murine ActRIIB consisting of amino acid residues 1 to 174 operably linked to the myosin light chain promoter and 1/3 enhancer, wherein

elevated levels of the truncated receptor result in increased muscle mass in the transgenic mouse as compared to a corresponding non-transgenic mouse, and methods of making such a transgenic mouse. It is alleged, however, that the specification does not enable all transgenic non-human animals or other truncated Activin Type II receptors expressed from other promoters.

It is stated in the Office Action, for example, the specification does not teach the generation of any animal other than transgenic mice. Applicants point out, however, that the claims as amended encompass only transgenic non-human mammals, and the specification discloses methods of producing such transgenic non-human mammals, referring, for example, to U.S. Pat. Nos. 6,140,552 and 6,218,596 (see paragraph 138, page 52-53). U.S. Pat. No. 6,140,552, for example, describes methods for making transgenic bovine species. In addition, paragraphs 140-143 of the specification disclose methods for generating transgenic non-human animals, including using microinjection of a pronuclear cell, by electroporation, plasmid transfection or microinjection of a transgene into an embryonic stem cell, by infection of an embryonic stem cell using a transgene-containing retrovirus and by cross-breeding chimeric animals. As such, it is submitted that the specification clearly teaches the generation of transgenic non-human mammals beyond transgenic mice.

Further, it is submitted that methods of making transgenic non-human mammals are known in the art. For example, U.S. Pat. No. 6,271,436 (a portion of which is attached as Exhibit A) describes cells and methods for generating transgenic pigs (see, e.g., Abstract; Example 4, columns 82-86; and Example 10, columns 104-107; see, also, claim 55, columns 181-182). In addition, U.S. Pat. No. 6,107,543 describes cells and methods for making bovines from such cells, including from such cells containing an exogenous nucleic acid molecule (Exhibit B, see, e.g., column 5, lines 28 claims 1 and 6). Also, U.S. Pat. No. 6,194,635 describes cells and methods for making chimeric and transgenic ungulates, particularly porcine species (Exhibit C, see Example 7, columns 13-18 - e.g., column 14, line 44, to column 15, line 49; see, also, Examples 8 and 9).

In support of this ground of rejection, the Office Action cites to various references as evidence that the generation of transgenic mammals is unpredictable with respect to transgene behavior. For example, Hammer et al. is cited as showing that the same transgene construct that elicited a phenotypic change in mice had no effect in sheep or pigs. Applicants point out, however, that this result of Hammer et al. was not "unpredictable" or unexpected because, as Hammer et al. indicate, it was known that human growth hormone, as compared to porcine growth hormone, "had only a weak growth-promoting effect in pigs (Baile et al., 1983)" (paragraph bridging pages 276-277). Thus, it was not unpredictable that expression of human growth hormone transgene in a porcine species would have minimal effect because such an effect of human growth hormone in pigs was known. Further in this respect, it is noted that Hammer et al. suggest that introducing a transgene encoding porcine growth hormone may enhance growth because it was known that injections of porcine growth hormone had such an effect (paragraph bridging pages 276-277). As such, it is submitted that, if anything, Hammer et al. provides evidence that the effect of transgene expression in a mammal can be predicted based, for example, on knowledge of the effect of the transgene product in the mammal.

Ebert et al. is cited as reporting that a transgenic pig containing a Moloney murine virus rat somatotropin fusion gene did not develop an expected phenotype of growth during the rapid growth phase. Applicants point out, however, that Ebert et al. do not report that there was no increase in "growth" during the rapid growth phase, but that there was no increase in "the rate of growth" (see Abstract). In fact, Ebert et al. state that "Skeletal growth was markedly increased and fat deposition was reduced throughout the animal" (Abstract; emphasis added). Moreover, Ebert et al. conclude that "Our studies indicate that major phenotypic changes can be produced in transgenic livestock through expression of microinjected fusion genes" (page 281, right column, second full paragraph). Thus, it is submitted that Ebert et al. demonstrate that transgene expression in livestock can produce a desired and expected effect.

Mullins et al. is cited as stating that "a given construct may react very differently from one species to another" (Summary). However, Mullins et al. go on to state, for example, that the application of transgenics in pigs should produce major advances in the fields of transfusion and transplantation (Summary). In addition, Mullins et al. indicate that various potential problems that may occur as a result of transgenic methods can be addressed, including, for example, pointing out that "Position-independent, copy-number related expression can be achieved" using a variety of promoter elements and that "Such elements have been shown to function across species barriers, and their incorporation into gene constructs can overcome position effects and improve expression of heterologous genes within specific cell types" (paragraph bridging pages 1557-1558). Further, Mullins et al. describe several successful applications of transgenic in mammals other than mice (see pages 1558-1559, section entitled "Nonmurine species in biomedical research"). As such, we would submit that the Mullins et al. reference, which was published in 1996 and does not contain any citation after 1995, does not necessarily support the position that undue experimentation would have been required for one skilled in the art to practice the claimed invention at the time the subject application was filed.

Wall is cited as reporting that transgene expression in the mouse is not always predictive of the physiological consequences of transgene expression in livestock. It is noted that the Examiner appears to be referring to Wall, Theriogenology 45:57, 1996, which does not appear to be of record in the present case. Nevertheless, Applicants have reviewed Wall (1996), and point out that Wall et al. (J. Dairy Sci. 80:2213, 1997) stated that the "Characteristics of transgene expression in transgenic goats, mice, pigs, rabbits, rats, and sheep appear to be similar. Although data are insufficient to characterize transgene expression for transgenic cattle, there is no reason to expect that transgenes behave differently in that species." (page 2216, left column, first full paragraph). As such, it is submitted that, after the publication of Wall (1996), and prior to the time the subject application was filed, Wall et al. (1997) considered transgene expression in mice to be reasonably predictable of transgene expression in other mammals.

McPherron et al. is cited as reporting that "Unlike in mice, a myostatin null mutation in cattle causes a reduction in sizes in internal organs and only a modest increase in muscle mass." Applicants point out that McPherron et al. are not describing "transgenic" cattle, but are reporting on naturally occurring cattle that have a mutation in the myostatin gene. Nevertheless, it is noted that the null mutation in cattle resulted in an increase in muscle mass, even if only modest and, in this respect, it is noted that the claims do not require any particular level of increased muscle mass. Further, McPherron et al. indicate that the more modest increase in muscle mass observed in the cattle may be due to cattle being nearer to a maximal size because of selective breeding processes (page 12460, left column, second full paragraph). Thus, even if the McPherron et al. reference is relevant to transgenic applications, it would not appear to support the position that transgene expression in mammals other than mice would produce an unpredictable result.

It is further stated in the Office Action that, while one skilled in the art can produce transgenic animals comprising a gene of interest, it is not predictable whether the transgene would function at a level and specificity to produce a particular phenotype. Hammer et al. is cited as discussed above. However, for the reasons set forth above, the results described by Hammer et al. in transgenic pigs was predictable because it was known that human growth hormone injections did not affect porcine growth. Similarly, Ebert et al. is cited as discussed above. However, for the reasons set forth above, Ebert et al. appear to support the predictability of transgene expression because expression of the somatotropin transgene resulted in "markedly increased" skeletal muscle. McPherron et al. also is cited as above. As discussed above, however, McPherron et al. do not describe results of transgenic mammals, but, instead, describe a breed of cattle having a mutation in the myostatin gene. As such, the reference would not appear to be relevant to the predictability of transgene expression.

Yamaoka et al. is cited as teaching that the Activin receptor may be dispensable for normal development of pancreatic islet cells, but that while redundancy may make a particular member of the TGF-β superfamily dispensable in some cases, such a result does not extend to all types of alterations in the TGF-β superfamily, or phenotypes produced by such alterations. For example, Yamaoka et al. is alleged to report that two alterations of TGF-β (i.e., over-expression of a TGF-β dominant negative, and a TGF-β knockout), which allegedly should result in the same phenotype, result in different effects on pancreatic acinar cells (citing to middle of page 300). However, a review of page 300 (and the entire Yamaoka et al. reference) did not find any mention of experiments relating to a TGF-β knockout phenotype. Instead, the cited passage appears to refer only to results obtained in an experiment using a TGF-β receptor dominant negative construct, and does not appear to provide any indication as to an unexpected or unpredictable phenotype observed due to expression of the dominant negative receptor. As such, Applicants are uncertain as to the allegedly inconsistent results referred to in the Office Action, and request clarification of this matter if the rejection based on this reference is not removed for the following reasons.

Notwithstanding the inability to find any discussion in Yamaoka et al. regarding phenotypes associated with a dominant negative as compared to a knockout transgenic animal, it is submitted that one skilled in the art would not necessarily expect these two different genetic alterations to result in the same phenotype because knockout of a gene results in a complete absence of expression of the "knocked out" gene product, whereas a dominant negative gene product, which is co-expressed in cells of a transgenic animal with the endogenous wild type gene product, competes with the wild type gene product. As such, in a situation such as that involving TGF-β superfamily members, in which redundancy can occur, it would not necessarily be unexpected or unpredicted that different phenotypes would be observed in a knockout animal as compared to a transgenic animal expressing a dominant negative gene product because a knockout results in complete loss of the gene product, whereas competition of a dominant

negative gene product and the corresponding endogenous gene product may not necessarily result in complete loss of the gene product function.

It is further stated in the Office Action that the specification fails to provide necessary guidance for muscle-specific promoters other than the myosin light chain promoter and the 1/3 enhancer. Wall is cited as stating that a lack of understanding of genetic control elements makes it difficult to design transgenes with predictable behavior, and Matthew et al: is cited as demonstrating that the level of expression the Activin Type II receptor ("Activin RII") is critical in generating a particular phenotype because the phenotype of *Xenopus* embryos was sensitive to the amount of Activin RII mRNA injected (i.e., "Activin RII expression"), and the level of expression correlated with a decrease in muscle actin expression.

Applicants point out, as mentioned above, that the claims do not require a specific amount of increased muscle mass in a transgenic non-human mammal of or made according to the present invention. As such, any increased muscle mass above that of a corresponding non-transgenic non-human mammal is sufficient to meet the requirements of the claims. In this respect, it is submitted that the results of Matthew et al. support the claimed invention because Matthew et al. report that an effect due to expression of the Activin RII correlates with the amount of receptor expressed; i.e., some effect can be expected at pretty much any level of expression of the Activin RII. Thus, in view of the results of Matthew et al., it is submitted that the skilled artisan, viewing the subject application, would have known that expression of a truncated Activin RII lacking kinase activity in a transgenic non-human mammal of the invention would result in increased muscle mass in the transgenic mammal, and that the amount of increased muscle mass would correlate (inversely) with the amount of truncated Activin RII expressed.

Applicants further point out that the specification discloses numerous regulatory elements useful for directing expression of a transgene comprising an operably linked nucleic acid

molecule encoding a truncated Activin RII lacking kinase activity (see, for example, paragraph 144, page 55). In addition, it is submitted that the skilled artisan would have known of numerous muscle-specific promoters, including, for example, the promoters of the chicken skeletal muscle α-actin gene (see Exhibit D, Abstract) and the pM promoter of the human aldolase A gene (see Exhibit E, Abstract). As such, the skilled artisan would have known how to express a transgene encoding a truncated Activin RII in cells of a transgenic non-human mammal, including in a muscle cell specific manner.

For the reasons set forth above, it is submitted that one skilled in the art, viewing the subject application, would have known how to make and use the claimed transgenic non-human mammals and methods of the invention without undue experimentation. Accordingly, it is respectfully requested that the Examiner reconsider and withdraw the objection to the specification, and remove the corresponding rejection of the claims under 35 U.S.C. § 112, first paragraph.

The rejections of claims 41 and 42 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter of the invention are respectfully traversed.

With respect to claim 41, it is noted int he Office Action that the term "wherein the progeny are hatched" is unclear in view of the subject matter presently under examination. Claim 41 has been amended to more clearly indicate that the method requires implanting the embryo into the oviduct of a pseudopregnant female of the same species, thereby allowing the embryo to mature to full term progeny. As such, it is respectfully requested that this ground of rejection be removed.

With respect to claim 42, it is noted in the Office Action that the term "the animal" lacks antecedent basis. Claim 42 as amended no longer contains the term "the animal" and, therefore, it is submitted that this rejection is moot.

It is submitted that the skilled artisan, reading the claims, would know the metes and bounds of the claimed invention. Accordingly, it is respectfully requested that the rejections under 35 U.S.C. § 112, second paragraph, be removed.

In view of the amendments and the foregoing remarks, it is submitted that the claims are in condition for allowance, and a notice to that effect is respectfully requested. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to the subject application.

The Commissioner is authorized to charge any additional fees, or made any credits, to Deposit Account No. 50-1355.

Respectfully submitted,

Date: July 3, 2003

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Enclosures: Exhibits A to E

EXHIBIT E

MCB Molecular and Cellular Biology

Mol. Cell. Biol., Jan 1993, 9-17, Vol 13, No. 1

An opportunistic promoter sharing regulatory sequences with either a muscle-specific or a ubiquitous promoter in the human aldolase A gene

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The human aldolase A gene is transcribed from three different promoters, pN, pM, and pH, all of which are clustered within a small 1.6-kbp DNA domain. pM, which is highly specific to adult skeletal muscle, lies in between pN and pH, which are ubiquitous but particularly active in heart and skeletal muscle. A ubiquitous enhancer, located just upstream of pH start sites, is necessary for the activity of both pH and pN in transient transfection assays. Using transgenic mice, we studied the sequence controlling the muscle- specific promoter pM and the relations between the three promoters and the ubiquitous enhancer. A 4.3-kbp fragment containing the three promoters and the ubiquitous enhancer showed an expression pattern consistent with that known in humans. In addition, while pH was active in both fast and slow skeletal muscles, pM was active only in fast muscle. pM activity was unaltered by the deletion of a 1.8-kbp region containing the ubiquitous enhancer and the pH promoter, whereas pN remained active only in fast skeletal muscle. These findings suggest that in fast skeletal muscle, a tissue-specific enhancer was acting on both pN and pM, whereas in other tissues, the ubiquitous enhancer was necessary for pN activity. Finally, a 2.6-kbp region containing the ubiquitous enhancer and only the pH promoter was sufficient to bring about high-level expression of pH in cardiac and skeletal muscle. Thus, while pH and pM function independently of each other, pN, remarkably, shares regulatory elements with each of them, depending on the tissue.(ABSTRACT TRUNCATED AT 250 WORDS)

This article has been cited by other articles:

- Hallauer, P., Bradshaw, H., Hastings, K. (1993). Complex fiber-type-specific expression of fast skeletal muscle troponin I gene constructs in transgenic mice. *Development* 119: 691-701 [Abstract]
- vanWert, J. M., Panek, H. R., Wolfe, S. A., Grimes, S. R. (1998). The TE Promoter Element of the Histone H1t Gene Is Essential for Transcription in Transgenic Mouse Primary Spermatocytes. *Biol Reprod* 59: 704-710 [Abstract] [Full Text]
- Spitz, F., De Vasconcelos, Z. A., Chatelet, F., Demignon, J., Kahn, A., Mira, J.-C., Maire, P., Daegelen, D. (1998). Proximal Sequences of the Aldolase A Fast Muscle-specific Promoter Direct

Nerve- and Activity-dependent Expression in Transgenic Mice. *J. Biol. Chem.* 273: 14975-14981 [Abstract] [Full Text]

- Spitz, F., Demignon, J., Demeurie, J., Sabourin, J.-C., Kahn, A., Daegelen, D., Maire, P. (1998). A Binding Site for Nuclear Receptors Is Required for the Differential Expression of the Aldolase A Fast-twitch Muscle Promoter in Body and Head Muscles. *J. Biol. Chem.* 273: 561-567 [Abstract] [Full Text]
- Sabourin, J.-C., Kern, A.-S., Grégori, C., Porteu, A., Cywiner, C., Châtelet, F.ço.-P., Kahn, A., Pichard, A.-L. (1996). An Intronic Enhancer Essential for Tissue-specific Expression of the Aldolase B Transgenes. *J. Biol. Chem.* 271: 3469-3473 [Abstract] [Full Text]
- Porcher, C., Picat, C., Daegelen, D., Beaumont, C., Grandchamp, B. (1995). Functional Analysis of DNase-I Hypersensitive Sites at the Mouse Porphobilinogen Deaminase Gene Locus. *J. Biol. Chem.* 270: 17368-17374 [Abstract] [Full Text]